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HISTOGENESIS OF THE RETINA[1]

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INTRODUCTION

THE histology of the vertebrate retina has been carefully investigated in several animals and the adult structure of that organ is now fairly well understood, but the development of the retinal elements has received very little attention. The literature on the eye deals chiefly with the formation of the optic vesicles and with the histology of the adult organ. The only writer who has attempted to follow out the histogenesis of the retinal elements appears to be Cameron (:05) in a series of papers on the development of the retina in Amphibia, while Bernard (:00- :04) has given some attention to the development of the rods and cones. The investigations on which the present paper is based, were made on the retina of the chick. This animal was selected for study, first because it has never been investigated before, and second because it affords so readily a complete series of stages of development so that there need be no gaps for lack of material. Since it has been necessary to compare the results obtained here with those of Cameron, his technique has been followed closely, but in addition certain modifications of it have been used, for while in many cases by using his methods results precisely similar to his have been obtained, yet by other methods results more in accord with the known processes of cell and tissue development in other organs of the body have been secured.

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[1] From the Biological Laboratory of Boston University, College of Liberal Arts.

Technique

In the course of this work several fixing reagents have been tried with varying degrees of success. Of these, Kleinenberg's picrosulphuric mixture has proved entirely satisfactory in most cases, but the best results have been obtained from the fluid used by Cameron and known as the Bles fluid; it is made as follows:—

70 % alcohol	90 parts
Glacial acetic acid	3 "
Commercial formalin	7 "

The embryos remained in this for one week and were then transferred to 70% alcohol.

When needed, the eyes were dissected out, cut in halves by a vertical section through the optical axis, and placed in 90 % alcohol for three hours, followed by 95 % alcohol for from six to twelve hours according to size. They were then cleared in cedar oil and finally imbedded in paraffin, being passed through two paraffin baths of one and three hours each, kept at a temperature of 53° C.

All sections were made with a Bausch and Lomb sliding microtome and mounted in the usual way. All were stained on the slide.

In most cases two different staining methods were used in each stage of development for the sake of comparison. These were a 33 % aqueous solution of Delafield's hæmatoxylin followed by an alcoholic solution of eosin, and an iron-alum preparation described by Cameron.

This second method gave entire satisfaction in every respect when modified by the subsequent use of eosin. The slide was first placed in a 4 % aqueous solution of iron-alum, the violet-tinted crystals, and allowed to remain ten minutes. This treatment acts as a mordant of course. It was then thoroughly washed in tap-water, dipped in a saturated aqueous solution of hæmatoxylin for ten minutes, and again washed in water. This left the sections jet black. The slide was then placed once more in the iron-alum solution and carefully watched until the sections were of a light purple tint. They were then rinsed in water and examined under the microscope. If over-stained they were bleached a little longer in the iron-alum, if not stained enough the hæmatoxylin was repeated. The process has the advantage that it admits of absolute control. The slide was next placed in an alcoholic solution of eosin for about fifteen seconds and the excess of stain washed out in alcohol. An oil-immersion lens is absolutely essential for making out the details of retinal development.

Earlier Stages of Retinal Development

After the primary optic vesicle has invaginated to form the secondary optic vesicle or optic cup, as shown in Fig. 1, it is readily seen that the invaginated portion, the earliest stage in the development of the retina, and the uninvaginated portion, which is continuous with it and is to become the pigment layer, consist of nuclei essentially similar in structure, with more or less granular protoplasm about them,—the two layers differing in thickness only. Karyokinesis at this stage takes place at the margins only of the optic cup, in both the retinal and the pigment layers.

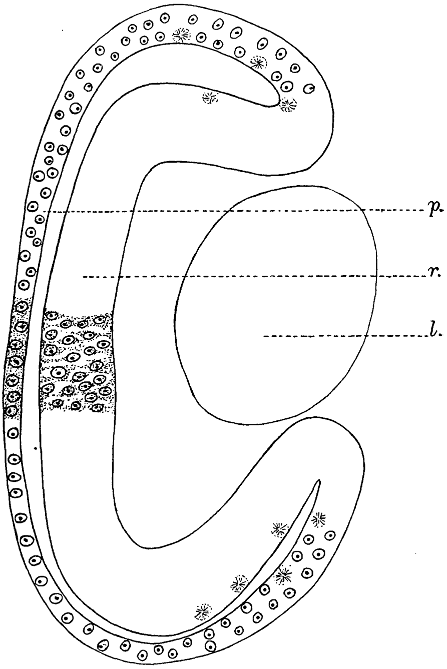

FIG. 1.— Section through the entire optic cup of a 64 hours' embryo showing relative distribution of the nuclei in the pigment layer and the retina; the mitotic figures indicate the only points at which karyokinesis takes place at this stage of development. x 220. *l.*, lens; *p.*, pigment layer; *r.*, retina.

In the earliest stage in which the retina as such is to be identified, its structure consists of nuclei suspended in cytoplasm without cell walls, *i. e.*, it is a syncytium whose outer and inner extremities form the external and internal limiting membranes. This is in accord with the conclusion of Bernard (:00). Early writers described it as made up of discrete cells, while Cameron goes to the opposite extreme and maintains that it consists of nuclei with absolutely no cytoplasm at all. Cytoplasm there certainly is in the chick at this stage if a cytoplasmic stain is used, but it is almost impossible to demonstrate it with a nuclear stain alone. It is difficult, however, to determine just what or how much cytoplasm is to be associated with each nucleus since cell walls are absent. The nuclei are evidently all alike except for slight differences in shape, some being elliptical and some circular in outline; this may be due to their being cut in different diameters. The first measure 7.29μ by 4.38μ, while the second are 5.83μ in diameter. This is as large as any retinal nuclei ever become.

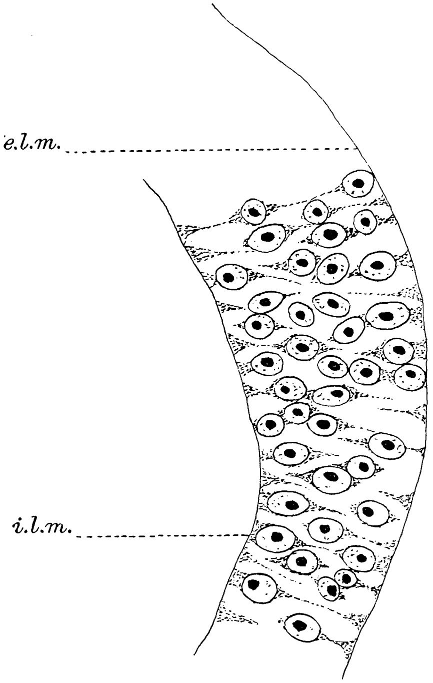

Fig. 2.— Section through a portion of the retina of a 56 hours' embryo. Each nucleus is provided with cytoplasm at its inner and outer ends. There is no cell division going on at this point in the retina at this stage. x 810. *e. l. m.*, external limiting membrane; *i. l. m.*, inner limiting membrane.

Although no structural difference is apparent, there is, however, an intrinsic difference in these nuclei. Those next the external limiting membrane have the power of division, while the others have not. These then may be called the row of germinal nuclei, which at this early stage are not dividing except at the junction of the retina with the pigment layer, but division begins immediately after the complete formation of the optic cup, so that karyokinetic figures are found from margin to margin until the beginning of differentiation of the retinal layers.

The dividing nucleus is surrounded by a clear, fluid-filled space several times as large as the other nuclei. Its outline, though formed by the surrounding protoplasm, is so pronounced as to simulate closely the appearance of an enveloping membrane. This is well shown in Fig. 5.[1] In the one in the center the plane of the section passes through the long axis of the spindle, while in the others it cuts through or parallel with the equator. Thus it is seen that the plane of division is always perpendicular to the external limiting membrane. No exceptions to this rule have

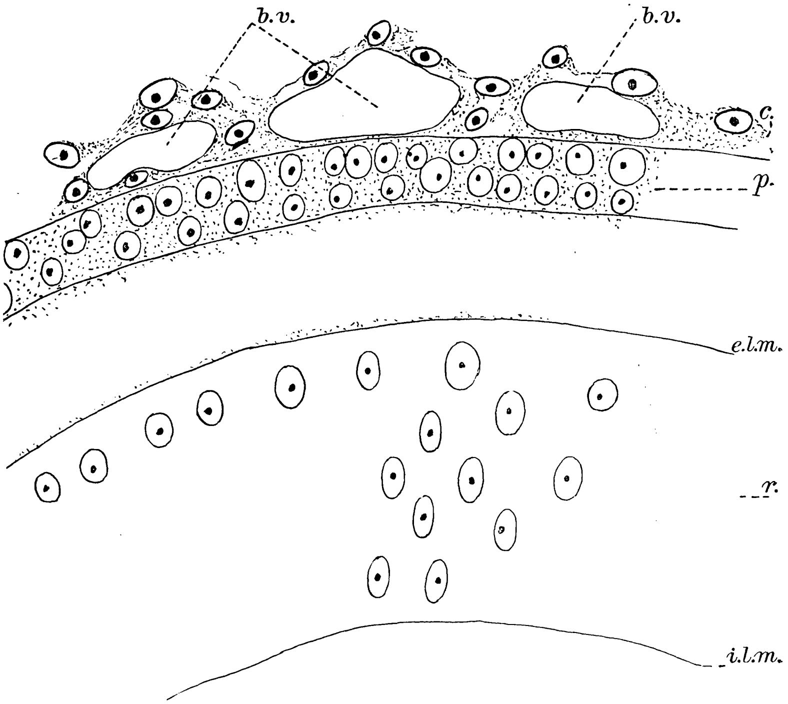

FIG. 3.— From a section of the eye of an 84 hours' embryo showing the numerous blood vessels in the choroid in close contact with the pigment layer, which here consists of two layers of nuclei embedded in a common cytoplasmic mass with no pigment granules. The wide separation of the external limiting membrane from the pigment layer is artificial. x 810. *b. v.*, blood vessel; *c.*, choroid; *e. l. m.*, external limiting membrane; *i. l. m.*, inner limiting membrane; *p.*, pigment layer; *r.*, retina.

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[1] All figures are from chick embryos and were drawn with the Abbé camera lucida to the magnification indicated in each case; in some figures the details

been observed. After each division into two, one nucleus is left behind to contribute one additional row to the thickness of the retina while the other grows to its original size and divides again. Thus the external limiting membrane is continually moving outward the width of a nucleus with each successive generation of germinal nuclei. The fate of the nuclei thus left behind will be considered in connection with the various retinal layers.

Differentiation begins at the time the majority of germinal nuclei at the center of the retinal cup cease to divide, that is, at the end of the period of most rapid growth. Commencing thus at the center it gradually encroaches upon the territory of

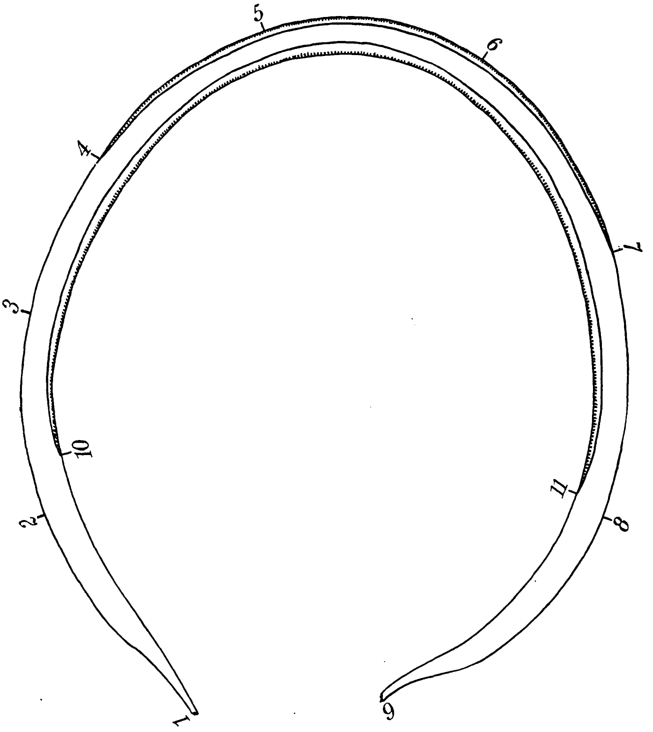

FIG. 4.— Outline drawing of a section of the entire retina of a 7¾ days' embryo. The layer of rods and cones and the outer reticular layer extend from IV to VII. The ganglion-cell layer and the inner reticular layer extend from X to XI. In the layer of germinal cells the numbers of nuclei in a dividing stage are as follows: from I to II, 82; from II to III, 47; from III to IV, 32; from IV to V, 17; from V to VI, 5; from VI to VII, 23; from VII to VIII, 56; from VIII to IX, 80. x 19.5.

the undifferentiated margins. In this way every section contains in its various parts all the preceding stages through which it has passed. For instance a segment near the margin of a ten days' retina would have attained the same degree of development as a segment through the center of the retinal cup from a younger eye. Hence in the discussion of development in this paper it is always the most highly developed portion of the retina, the center, on which all conclusions are based, and not the parts nearer the margin, which invariably represent an earlier stage.

In the growth of the retina as a whole there are three well defined periods: (1) the period of cell-multiplication, (2) the period of readjustment, (3) the period of final differentiation. The first, which extends from the second to the eighth day, is characterized by a tremendous increase in the number of nuclei and consequently in a rapid growth in the size and thickness of the whole retina, which at the end of this stage has attained its maximum width of 195 μ. During the succeeding period of readjustment, from the eighth to the tenth day, there is still a rapid growth at the margins, but at the center of the retinal cup the number of nuclei is henceforth practically fixed, and the principal change is that of redistribution or readjustment into layers. Incidental to this there is a sudden decrease in width to 150 μ, undoubtedly due to a stretching out of the surface area. The stage of differentiation, extending from this time to the end of incubation, is marked by the growth of cytoplasmic processes and by a gradual increase to 180 μ in the thickness of the retina.

THE GANGLION-CELL LAYER

The ganglion-cell layer is the first to appear with the beginning of the period of readjustment, as shown in Fig. 6. It consists at first of three rows of nuclei which are marked off from the others by the commencement of the inner reticular layer. As the surface area of the retina increases, these nuclei, whose number of course remains the same,[1] gradually fall into line, beginning at the center of the retinal cup and thence radiating outwards on every side toward the margins, until at the end of this period the layer comes to be composed of a single phalanx of nuclei (Fig. 7). This readjustment takes place in the direction of the internal limiting membrane as is shown by the fact that there is no corresponding increase in the width of the nerve-fiber layer. Thus in its development the ganglion-cell layer actually decreases in width from 28.6 μ to 10.4 μ.

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[1] In a 7¾ days' embryo a single dividing nucleus was found in this layer, the only exception in the entire series studied.

Part of the nuclei of this layer are the same nuclei that were seen in the early retina immediately after the formation of the optic cup (Fig. 1), and the remainder are the first nuclei to be formed by the division of the germinal nuclei. All are of the same size as those found in the early undifferentiated condition; that is to say, after division each grows to the size of the parent nucleus, a fact also true of the amacrines or horizontal nuclei. At the advent of the third stage of growth, with its attendant increase in the amount of cytoplasm, these cells become multipolar and gradually approach nearer and nearer the condition of the adult retina.

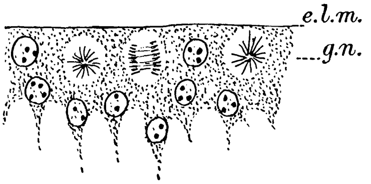

Fig. 5.— Germinal nuclei in process of division,— from the retina of a 6½ days' embryo. All the nuclei are imbedded in a mass of granular cytoplasm, and in each case the cleavage plane is at right angles to the external limiting membrane. x 1500. *e. l. m.*, external limiting membrane; *g. n.*, germinal nuclear layer.

The Inner Nuclear Layer

The inner nuclear layer, which is by far the largest and most conspicuous of all, has its beginning in the fourteen successive generations of nuclei immediately following the production of those which are eventually to become the ganglion cells. Fig. 12 shows this layer in the process of formation and Fig. 13 gives the appearance three fourths of a day later. It does not become a complete layer by itself until the beginning of the period of readjustment when the outer reticular layer appears and separates it from the future external nuclear layer; but long before this, differentiation has already taken place.

In this layer the nuclei of the first generation become the inner horizontal cells while the nuclei of the last generation become the outer horizontal cells. In both cases, as mentioned before, these nuclei after division grow to the size of their immediate ancestors, the early germinal nuclei. This, however, is not true of the intermediate generations. Here each successive phalanx fails to attain quite the size of the one preceding, so that when the process is complete we get the effect of a gradation of nuclei apparent in Fig. 15, where they range in size from 5.83 μ to 4.38 μ in diameter.

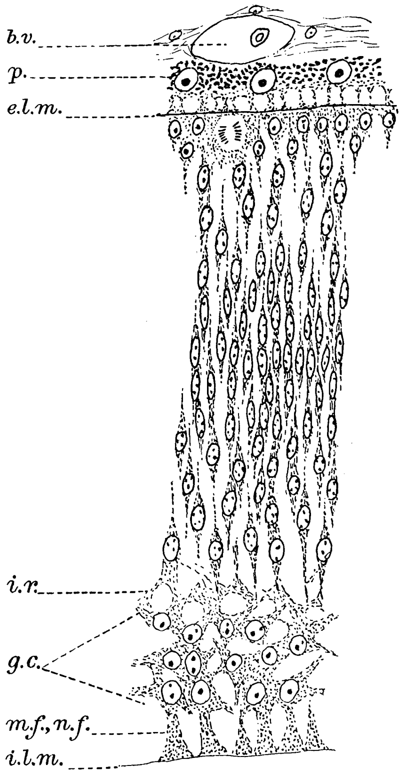

FIG. 6.— Section through the retina of a 7¾ days' embryo. The ganglion-cell layer here consists of three layers of nuclei and the inner reticular layer has just begun to form. Nuclei are still dividing in the germinal layer, and the pigment layer is connected with the external limiting membrane by cytoplasmic strands. x 440. *b. v.*, blood vessel; *e. l. m.*, external limiting membrane; *g. c.*, ganglion-cell layer; *i. l. m.*, inner limiting membrane; *i. r.*, inner reticular layer; *m. f.*, radial fibers of Müller; *n. f.*, nerve-fiber layer; *p.*, pigment layer.

During the first two periods of growth the nuclei of this layer, which measure 7.29 μ by 2.92 μ on an average, are all extremely elliptical in outline, with the long axis at right angles to the limiting membranes. When examined with a low magnifying power they appear sharply pointed at the ends, and Bernard (:04) has figured them thus in his Plate 29, Fig. 25. But when studied with the oil immersion these nuclei are seen to be elliptical and bounded by a very distinct membrane which is rounded at the ends. They present very much the appearance of having been pulled outward toward the layer of germinal nuclei, for the cytoplasm about them streams from each pole in the direction of the limiting membranes. Some of these nuclei, especially those near the middle of the layer, eventually become supporting cells or fibers of Müller. This is well shown in Fig. 12. Up to the end of the period of readjustment the chromatic substance of all retinal nuclei is scattered about in the nucleus as several granules; later, there is a single large, clearly defined chromatic mass. This condition appears to be true of other embryonic tissues as will be seen by the figure, but it is especially evident in these elliptical nuclei of the inner nuclear layer.

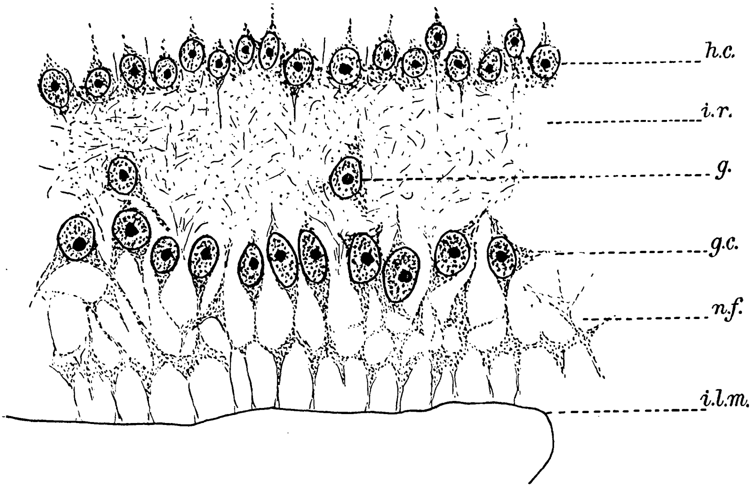

Fig. 7.— Portion of the retina of a 10 days' embryo. The ganglion-cell layer now consists of two layers of nuclei. The inner reticular layer is well differentiated and the adjacent nuclei of the inner nuclear layer will develop chiefly into horizontal cells. x 810. *g.*, ganglion cell; *g. c.*, ganglion-cell layer; *h. c.*, horizontal cells or amacrines; *i. l. m.*, inner limiting membrane; *i. r.*, inner reticular layer; *n. f.*, nerve-fiber layer.

To account for the enormous increase in the number of these nuclei in so short a period two explanations have been advanced: the theory of migration and the theory of direct division; neither of these appears to us probable. The first is advanced by Bernard (:00–:04). He states that in order to account for this rapid increase in numbers "we have to assume a stream of nuclei from the undifferentiated edges of the retina towards the base of the cup." In another place in the same paper he asks the question: "Where does the middle nuclear layer get its supply of nuclei to furnish the outer nuclear layer?" Our work on the chick demonstrates clearly that the outer nuclear layer is not derived from the inner nuclear layer in any sense of the word. Each layer is first laid down by successive generations of nuclei from within outward as Fig. 12 shows. As before stated this process is followed by a later readjustment in each layer, but not by migration from one layer to another. As for Cameron's theory of direct division there is absolutely no evidence that such a process takes place in the chick. Even at the center of the retinal cup mitotic division does not wholly cease until nearly all the rods and cones have reached an advanced stage of development.

From the time the outer reticular layer begins to appear at the end of the first period of growth no more nuclei are added to the inner nuclear layer. It has then attained its maximum thickness of 111.8 μ. The nuclei are all crowded very closely together, this being especially true of the half of the layer nearer the external limiting membrane. Then begins the period of readjustment, during which the layer diminishes in thickness to 65 μ. With the stretching out in area of the whole retina and its consequent restoration of equilibrium or equalization of tension, if we may so express it, the nuclei become more loosely arranged and gradually assume the circular outline, Figs. 13 and 15. The number of phalanges, or rows of nuclei, which was at first fourteen, has now decreased to eight, for there has been a gradual closing up of the ranks in the direction of the external limiting membrane, made evident by the corresponding increase in the width of the inner reticular layer.

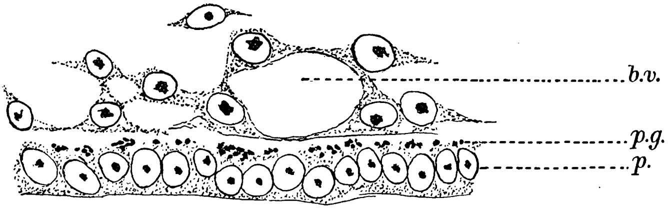

FIG. 8.— An 88 hours' embryo, in which pigment granules appear in the outer portion of the pigment-layer cells; the nuclei of this layer are closely packed together. x 810. *b. v.*, blood vessel; *p.*, pigment layer; *p. g.*, pigment granules.

In the final stage of differentiation there is little change in this layer except for a slight decrease in thickness from 65 μ to 44.2 μ. The nuclei stain more deeply with the iron-alum hæmatoxylin, and all are spherical except those of the supporting cells or fibers of Müller, while the nuclei of the horizontal cells are readily distinguished by their clearer texture. The cytoplasm of the layer remains the same in amount and appearance as at the beginning.

THE RETICULAR LAYERS

The reticular layers are purely cytoplasmic in both origin and structure, there being no evidence to the effect that nuclei have anything to do with their formation. Each retinal nucleus, except in mitosis, is always enveloped in an intact nuclear membrane, and there are never appearances that might suggest the extrusion of nuclear substance or in the strict sense of the word the protrusion of processes of any kind. To be sure, the cytoplasmic processes of the bordering nuclei eventually extend into these layers to varying depths, but there is no ground for the assumption that such cytoplasm is of nuclear origin as Cameron believes is the case in the frog.

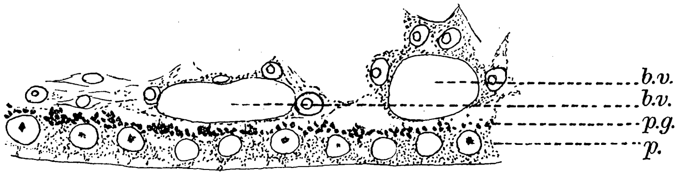

FIG. 9.— A 7 days' embryo; the nuclei of the pigment layer are more widely separated from each other; the pigment granules are more numerous and still entirely on the outer side of these nuclei. x 810. *b. v.*, blood vessel; *p.*, pigment layer; *p. g.*, pigment granules.

The inner reticular layer, which is the first of these to form, begins to appear at about the middle of the period of cell multiplication. It starts as a narrow protoplasmic rift between the third phalanx of the early ganglion-cell layer and the first generation of nuclei in the future inner nuclear layer (Fig. 6). This rift gradually widens, a little in the direction of the ganglion-cell layer, but chiefly toward the inner nuclear layer as these two layers develop. Viewed as a whole the layer has in section a crescent-shaped outline, and as it develops the pointed edges of the crescent encroach more and more upon the undifferentiated margins, as seen in Fig. 4.

The rate of development of this layer is fairly uniform from the time of its first appearance to the end of incubation. At the commencement of the period of readjustment it has attained a width of 13 μ, which has increased to 18.2 μ at its close. As will be remembered, it is during this time that the ganglion-cell nuclei fall into line, hence some of these stragglers are still to be seen lingering behind in this layer (Fig. 7). These are the nuclei which Löwe ('78) and Falchi ('87) found it so difficult to account for. From the beginning of the stage of differentiation onwards there is a constant increase in width up to 44.2 μ which is the final thickness of the inner nuclear layer.

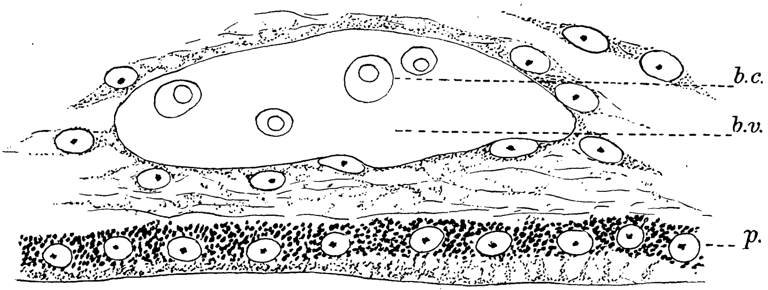

FIG. 10.— An $8\frac{1}{3}$ days' embryo in which the pigment granules now fill the cytoplasm between the nuclei but do not yet occupy the cytoplasm between the external limiting membrane and these nuclei. x 810. *b. c.*, blood cell; *b. v.*, blood vessel; *p.*, pigment layer.

Up to the stage of completed development the structure of this layer is practically homogeneous throughout, but as differentiation proceeds, vacuoles appear next the ganglion-cell layer so that the appearance is like that in Fig. 15. Later the processes from the ganglion cells and the cytoplasmic strands from nuclei of the inner nuclear layer can be traced to varying depths, while the fibers of Müller extend perpendicularly across the layer from the internal limiting membrane to the membrane of Henle.

The outer reticular layer begins to develop four or five days later than the inner and toward the close of the period of cell multiplication. Viewed as a whole it presents in section the same crescentic appearance, but the horns of the crescent extend into the undifferentiated region only half as far as those of the inner reticular layer (Fig. 4). Its width never increases but remains at 5.2 μ, though the boundaries are not so clearly defined at first and some straggling nuclei of the inner nuclear layer are still to be found in it up to the beginning of the stage of differentiation. At this stage the membrane of Henle is formed by the flattening of the inner ends of the rod and cone cells against the cytoplasm of the horizontal cells and against the ends of the fibers of Müller and of the processes from the inner nuclear layer (Fig. 13).

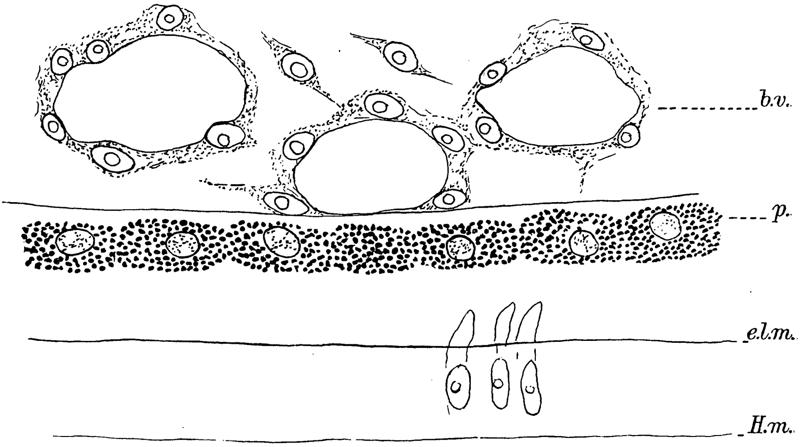

FIG. 11.— A 16½ days' embryo showing the cytoplasm of the pigment layer completely filled with pigment granules. x 810. *b. v.*, blood vessel; *e. l. m.*, external limiting membrane; *H. m.*, membrane of Henle; *p.*, pigment layer.

THE PIGMENT LAYER

The retina and the pigment layer, having as they do a common origin, develop in physical contact from margin to margin, and perhaps have an even more intimate connection, for the pigment layer probably plays an important *rôle* in the transfer of nutritive fluids to the multiplying nuclei and growing processes of the retina.

The pigment layer consists at first of two rows of closely packed nuclei, each 5.83 μ in diameter and surrounded by protoplasm which completely fills all the remaining space between the bounding membranes. The appearance is much like that suggested by Fig. 3. It is a significant fact that even at this early stage there are always numerous large blood vessels in the choroid tissue just outside the pigment layer.

It will be noticed in Fig. 1 that growth of the pigment layer is

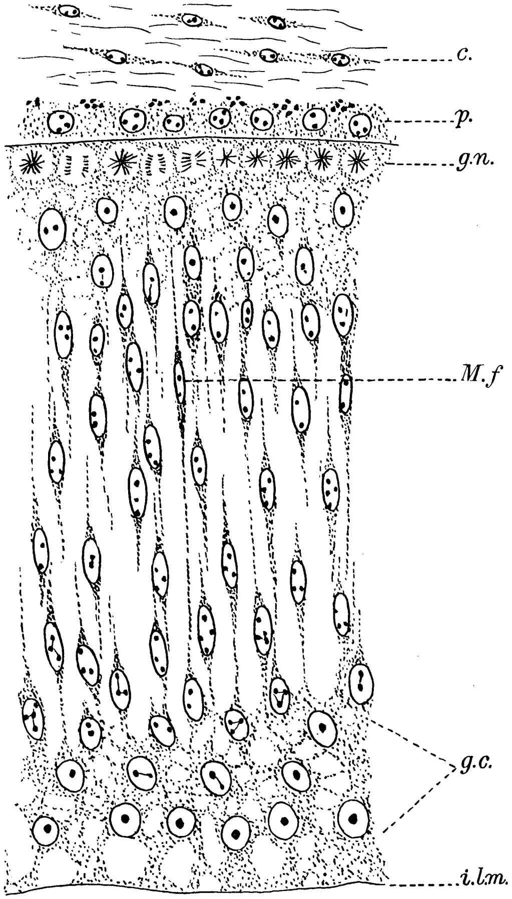

FIG. 12.— Section through the retina of a 7 days' embryo. The three layers of nuclei which will later become the ganglion-cell layer can be identified, as well as the bipolar cells of the inner nuclear layer, and the nucleus of a fiber of Müller is very distinct. Every nucleus of the germinal layer shows mitotic figures, the plane of division in each case being at right angles to the external limiting membrane; these nuclei are all imbedded in granular cytoplasm. x 810. *c.*, choroid; *g. c.*, ganglion-cell layer; *g. n.*, germinal nuclear layer; *i. l. m.*, inner limiting membrane; *m. f.*, radial fibers of Müller; *p.*, pigment layer.

at the margins and that even as early as two and two thirds days the surface area at the center of the cup has stretched out to such an extent that the nuclei here have fallen into line so that the layer consists of a single row of nuclei. The condition at the margin of the optic cup remains the same as long as the eye continues to increase in size. Hence the row of actively dividing nuclei is always relatively short compared with that of the retina of which it is a direct continuation. After division the resulting nuclei invariably grow to the size of the original nucleus, so there is never any apparent diminution in their size.

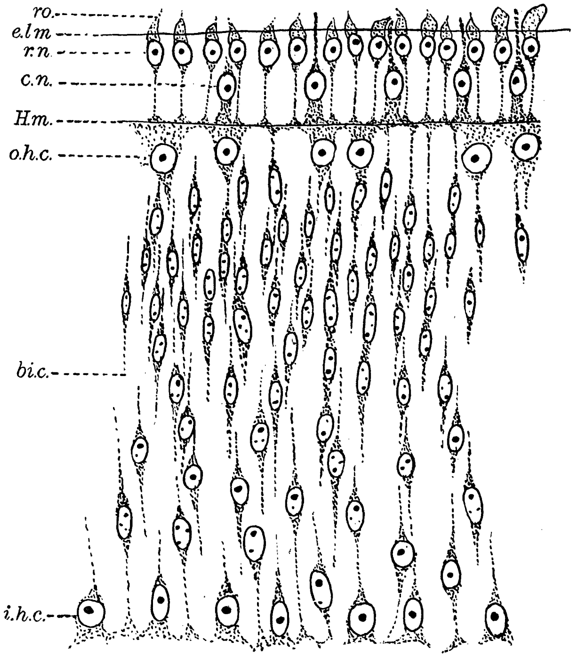

FIG. 13.— The retina of a 10½ days' embryo showing the structures outside of the inner reticular layer. In the inner nuclear layer can be distinguished the inner horizontal cells, the bipolar cells, the nuclei of the fibers of Müller and the outer horizontal cells. The outer reticular layer is forming, the membrane of Henle is distinct, and the outer nuclear layer shows the nuclei of the cones and of the rods in two layers. The cones appear as rather broad projections beyond the external limiting membrane and the cones as narrow cytoplasmic processes. x 810. *bi. c.*, bipolar cell; *c. n.*, cone nuclei; *e. l. m.*, external limiting membrane; *H. m.*, membrane of Henle; *i. h. c.*, inner horizontal cells; *o. h. c.*, outer horizontal cells; *ro.*, rods; *r. n.*, rod nuclei.

Pigment commences to form as early as three and two thirds days. Contrary to the statement which Cameron makes for the frog, the granules begin to appear in the chick on the side of the layer away from the retina and in the protoplasm between the nuclei, which shows them to be of cytoplasmic origin (Figs. 8 and 12). Cameron says that in the frog they appear first on the side adjoining the retina. As development proceeds and the nuclei become farther apart these granules gradually fill all the cytoplasm of the pigment layer on the side farthest from the retina (Fig. 9). It will also be noticed that at the same time vacuoles begin to appear in the undifferentiated cytoplasm invariably present between the pigment layer and the external limiting membrane of the retina (Figs. 6 and 10). This cytoplasm is very minute in amount at an early stage of development and later increases greatly, taking the form of strands which are the beginnings of the future rods and cones and of the cytoplasmic streamers which finally develop from the pigment layer and extend inward

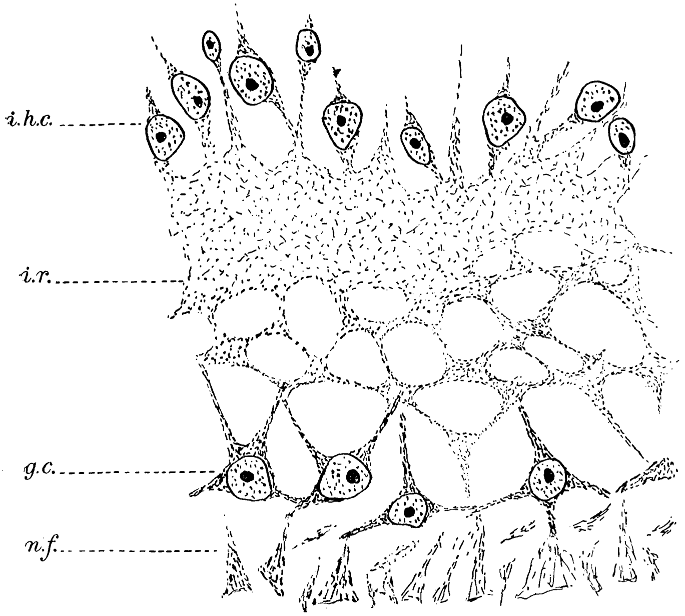

FIG. 14.— Inner portion of the retina of a 16⅓ days' embryo. The cells of the ganglion-cell layer now lie practically in a single plane. The inner reticular layer shows the large meshwork of its inner portion and the finer structure of its outer portion. x 810. *g. c.*, ganglion-cell layer; *i. h. c.*, inner horizontal cells; *i. r.*, inner reticular layer; *n. f.*, nerve-fiber layer.

between them. Meanwhile the cytoplasm about the nuclei becomes differentiated to such an extent that by sixteen and one third days the layer is seen to be composed of clearly defined cells each completely filled with pigment granules (Fig. 11). Later all finer structure is entirely obliterated by the pigment, but no granules are ever found normally outside their enveloping cytoplasm. The significance of these facts in the development of the retina is seen in connection with certain theories which Bernard has elaborated. He believes that pigment is a nutritive substance which is constantly being ingested and absorbed by the growing retina. This theory had its origin in Miss Huie's article on Drosera, in which she has succeeded in establishing the fact of an intracellular digestion. Bernard goes so far as to describe streams of absorbed pigment stretching through the inner nuclear layer and forming the fibers of Müller. Cameron has accepted this theory and applied it in his study on the amphibian retina. The nuclei, he believes, put forth an unformed ferment or enzyme under the action of which the pigment granules are rendered available for the metabolism of the cell. In this way the rod and cone vesicles grow by successive digestions.

Before the theory is accepted, however, it must be shown that pigment is actually a food substance and not as generally supposed a waste product. The theory must be harmonized, too, with the fact that pigment granules are extremely resistant to the action of all known ferments or digesting fluids as Cameron himself states. A further objection to the theory lies in the fact that in the chick these granules first form on the side of the pigment layer away from the retina, so that the rods begin to develop before there is any pigment in their vicinity. If they are able to begin to grow without it, is it not at least possible that they might continue to develop without it? Further, pigment is never found outside the protoplasm of the pigment cell; none ever appears in the retina, or even in contact with the rods and cones during their development. Besides, may not the mediation of a third substance and in such an unavailable form be entirely superfluous, for cannot the rods and cones obtain nutritive material for their growth direct from the numerous blood vessels just outside the pigment layer?

THE OUTER NUCLEAR LAYER

The outer nuclear layer, which forms during the period of readjustment, consists of two rows of nuclei. The one first laid down and adjoining the membrane of Henle is the layer of cone nuclei, which are fewer in number and more scattered in position (Fig. 13). The other, which borders on the external limiting membrane,

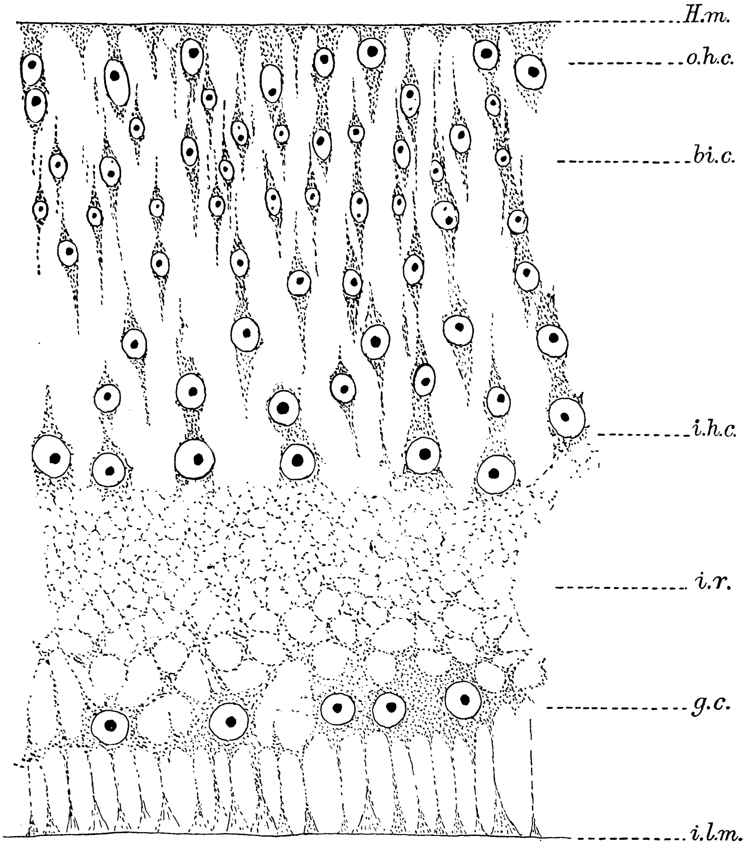

FIG. 15.— Retina of a 16⅓ days' embryo from the internal limiting membrane to the membrane of Henle. The expanded inner ends of the radial fibers of Müller are evident. The large nuclei of the inner and outer horizontal cells appear in the inner nuclear layer, the remaining nuclei in this layer belonging to bipolar cells and to the fibers of Müller. x 810. *bi. c.*, bipolar cell; *g. c.*, ganglion-cell layer; *H. m.*, membrane of Henle; *i. h. c.*, inner horizontal cells; *i. l. m.*, inner limiting membrane; *i. r.*, inner reticular layer; *o. h. c.*, outer horizontal cells.

represents the last product of the division of the germinal nuclei. In fact, division among them does not wholly cease until the rods of their immediate neighbors have attained a high degree of development. Thus the rod nuclei are the youngest in the retina. All measure 4.38 μ in diameter, which is the regular size of the germinal nuclei after the first half of the first period of growth. They suffer no diminution in size with the development of the rods and cones, and least of all do they receive any accessions to their number by a migration of nuclei from the inner nuclear layer.

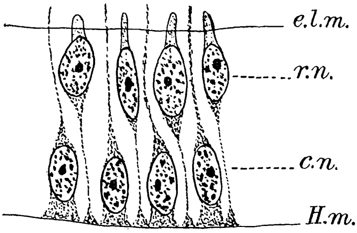

FIG. 16.— The development of the rods and cones in a 10 days' embryo. The rod-body is distinctly granular; the cone-body appears as a narrow cytoplasmic thread. x 1500. *c. n.*, cone nuclei; *e. l. m.*, external limiting membrane; *H. m.*, membrane of Henle; *r. n.*, rod nuclei.

Long before division has ceased in the row of rod nuclei, in fact as soon as the outer nuclear layer begins to appear at the center of the retinal cup, the rudiments of the future rods and cones can be made out in the shape of cytoplasmic threads or strands between the pigment layer and the external limiting membrane. The rods and cones are clearly of cytoplasmic origin. This cytoplasm slowly increases in amount so that at the beginning of the stage of differentiation the appearance is like that shown in Fig. 13. It will be noticed that a fragment of the original protoplasmic thread still tips the ends of some of the rod rudiments, while the base of the rod appears drawn out into a finely attenuated stalk whose inner extremity flattens against the membrane of Henle. The base of the cone is shorter and much broader (Fig. 16). As development proceeds, little or no change takes place in the outer nuclear layer strictly so called except that the nuclei toward the close of the period of differentiation stain more deeply with the iron-alum hæmatoxylin. This is true of all retinal nuclei.

The cytoplasm of the rods, which at first is evenly granular and homogeneous throughout, gradually increases in amount and assumes more and more the conical appearance presented in Fig. 17. About the middle of the period of differentiation, a clear unstaining vesicle appears at the tip or outer segment and persists in the same size and shape during the remainder of the development of the rod. There is no evidence that it is of nuclear origin in the chick, although Cameron describes it as such in the frog. The cytoplasm between this vesicle and the rod nucleus gradually elongates as development proceeds but without any appreciable increase in amount since at the same time the diameter diminishes, and eventually a conical cap of protoplasm develops on the distal end so that the unstaining vesicle appears like a fluid-filled vacuole imbedded in the rod about a fourth the way from the finely tapering point.

An interesting statement in this connection is made by Bernard, and repeated in more elaborate form by Cameron, to the effect that the nuclei of the rods tend to become protruded to varying degrees beyond the external limiting membrane. This certainly does not seem to be the case in the chick, although at first glance the appearance is strikingly in accord with such a statement. In the base of many of the rods and just outside the external limiting membrane appears a structure that might easily pass for a nucleus. The object proved a puzzle for a time, but with more careful study its explanation became clear. It was found that all the rods do not stand exactly perpendicular to the external limiting membrane, but many of them are bent over at various angles with it; and consequently these structures that look so much like nuclei are the truncated ends of other rods that once pointed toward the observer. By

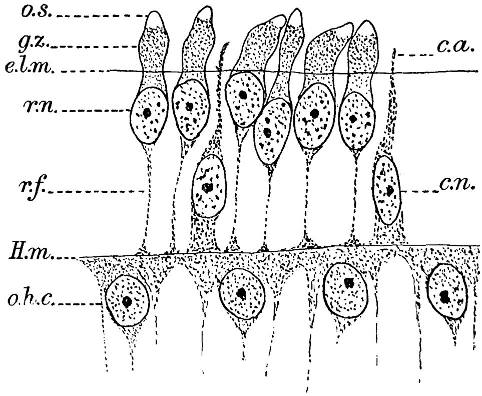

FIG. 17.— The developing rods and cones in an 11 days' embryo. The rods now show a clear outer segment and a granular inner zone. The cones are essentially the same as in the preceding figure. x 1500. *c. a.*, cone anlage; *c. n.*, cone nuclei; *e. l. m.*, external limiting membrane; *g. z.*, granular zone of rod; *H. m.*, membrane of Henle; *o. h. c.*, outer horizontal cells; *o. s.*, outer segment of rod; *r. f.*, rod fiber; *r. n.*, rod nuclei.

focusing down on them the remainder of the stump can be traced to its own nucleus. Hence the rod nucleus invariably retains its early position wholly inside the external limiting membrane. We have been unable to find such effects in the chick as Cameron figures in the frog in his Pl. 51, Figs. 24 and 29, where the nucleus forms a distinct projection beyond the limiting membrane.

VARIATIONS IN THE RELATIVE RATE OF DEVELOPMENT

In the development of the retina the time of appearance of the different elements is not absolutely fixed. An eye at ten days may have reached the same degree of development as another at twelve, or again it may be no further advanced than some that are two days younger. In the same retina it might be expected that the state of development of one layer would bear some definite relation to that of the others. But this is not the case. When the ganglion-cell layer has reached the stage shown in Fig. 6, the inner nuclear layer may appear as shown in that figure, or it may be more or less highly developed. Very frequently the inner nuclear layer reaches a very advanced stage before any cytoplasmic changes in the outer nuclear layer have begun to appear at all. On the other hand the rods and cones may be developing rapidly while the other layers are still in the earlier stages. The fact has been mentioned before that when the majority of the rods are in an advanced stage of development others are found beside them whose nuclei have just ceased dividing and whose rudiments have not begun to develop at all. Hence from the appearance of a part it is impossible to predict the stage of development of any other, for there appears to be no definite developmental ratio that might serve as a criterion. The drawings in each case are from the more typical representatives, and give the appearance most frequent at the specified age.

SUMMARY AND CONCLUSIONS

1. The retina consists at first of a syncytium.
2. Most of these nuclei eventually go to form the ganglion-cell layer; those next the external limiting membrane become the germinal nuclei.

3. Only the row of germinal nuclei has the power of division.

4. There are three well defined periods of growth: (*a*) the period of cell multiplication, second to eighth day; (*b*) the period of readjustment, eighth to tenth day; (*c*) the period of final differentiation, tenth day to end of incubation.

5. Up to the end of the first period the retina grows from within outward by the deposition of an additional row of nuclei with each successive generation.

6. After this, karyokinetic figures are found only at the margins.

7. Differentiation begins at the center of the retinal cup and gradually spreads in every direction toward the growing margins.

8. Between the center of the optic cup and the growing margin of any given retina are represented all the different stages through which it has passed; the nearer the margin, the younger the stage.

9. The ganglion-cell layer consists at first of three rows of nuclei.

10. These fall into line in the direction of the internal limiting membrane so as eventually to form but one layer.

11. In the inner nuclear layer differentiation into horizontal cells, fibers of Müller, and bipolar cells takes place *pari passu* with the formation of these nuclei.

12. This layer consists at first of about fourteen generations of nuclei.

13. With the exception of the horizontal cells each successive generation fails to attain quite the size of the one preceding.

14. As development proceeds the number of rows of nuclei decreases from fourteen to eight by a closing up of the ranks in the direction of the external limiting membrane.

15. Up to the end of the period of readjustment the nuclei of this layer are elliptical in outline with the long axis at right angles to the external limiting membrane.

16. Later these nuclei become circular in outline.

17. The reticular layers are cytoplasmic in both origin and structure.

18. The pigment layer is a direct continuation of the retina.

19. Like the retina its early structure is also a syncytium.

20. The nuclei at first are in two rows which early become arranged as one with the stretching out of the surface area.

21. Active growth is restricted to the margins of the pigment layers and continues as long as the eye increases in size.

22. Nuclei of this layer are always of the same size as those found in the early undifferentiated condition.

23. Pigment granules first form on the side of the layer away from the retina and in the protoplasm between the nuclei.

24. These granules are never normally found outside the cytoplasm of the pigment cell.

25. There is no evidence that pigment is a food substance.

26. The numerous large blood vessels always present in the choroid coat next the pigment layer may furnish the nutritive material for the development of the retina.

27. The outer nuclear layer represents the last two generations from the division of the germinal nuclei.

28. The rod nuclei are the youngest in the retina.

29. They are more numerous than the cone nuclei, and division among them does not wholly cease until the rods of their immediate neighbors have attained a high degree of development.

30. The rods and cones have their origin in undifferentiated cytoplasm.

31. There is no evidence that any part of the rod or cone is of nuclear origin.

32. The nuclei of the rods and cones retain their early position wholly within the external limiting membrane and do not tend to become protruded to varying degrees beyond it, as has been recorded for other animals.

33. In the development of the retina there is no fixed time for the appearance of the different elements.

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BIBLIOGRAPHY

BABUCHIN.

'65. Vergleichend-histologische Studien, nebst einem Anhang zur Entwicklungsgeschichte der Retina. *Würzburg. naturw. Zeitschr.*, vol. 5, pp. 127–143, pl. 4.

BAMBEKE, C. VON.

'79. Contribution à l'histoire du développement de l'oeil humain. *Ann. Soc. Méd. Gand,* pp. 1–22.

BARKAU, A.

'66. Beiträge zur Entwicklungsgeschichte des Auges der Batrachier. *Sitzb. Wien. Akad.*, vol. 54, pp. 70–74, pl. 1.

BERGMEISTER, O.

'80. Beiträge zur Entwicklungsgeschichte des Säugethierauges. *Mitth. embryol. Inst. Univ. Wien,* vol. 1, pp. 63–84, pls. 6–7.

BERNARD, H. M.

:00–04. Studies in the Retina. *Quart. Journ. Micr. Sci.*, vol. 44, pp. 443–469, pls. 30–31; vol. 46, pp. 25–76, pls. 3–5; vol. 47, pp. 302–363, pls. 27–29.

BRASS, ARNOLD.

'97. Atlas of Human Histology. London, 160 pp., 60 pls.

CAMERON, JOHN.

:01. The Movements of the Processes of the Retinal Pigment Cells. *Proc. Scott. Micr. Soc.*, pp. 219–225, pl. 18.

:05. The Development of the Retina in Amphibia. *Journ. Anat. and Phys.*, vol. 39, pp. 135–153, 332–348, 471–488, pls. 21, 32, 40–42, 51–52.

DOGIEL, A. S.

'83. Die Retina der Ganoiden. *Arch. f. mikr. Anat.*, vol. 22, pp. 419–472, pls. 17–19.

'92. Ueber die nervösen Elemente in der Retina des Menschen. *Arch. f. mikr. Anat.*, vol. 38, pp. 317–344, pls. 19–22.

EVETZKY, T. VON.

'79. Beiträge zur Entwicklungsgeschichte des Auges. *Arch. f. Augenheilk.*, Wiesbaden, vol. 8, pp. 305–356, pl. 3.

FALCHI, F.

'87a. Sull' istogenesi della retina e del nervo ottico. *Ann. Ottalm. Pavia,* vol. 15, pp. 528–536.

'87b. Sur l'histogénèse de la rétine et du nerf optique. *Arch. Ital. Biol.*, vol. 9, pp. 382–399, pl. 1.

GUNN, R. M.

'88. The Embryology of the Retina of Teleosteans. *Ann. Mag. Nat. Hist.*, ser. 6, vol. 2, pp. 263–269.

HIS, W.

'90. Histogenese und Zusammenhang der Nerven-elemente. *Arch. f. Anat. u. Phys.*, Suppl., pp. 95–118, 30 figs.

HUIE, LILY.

'96. Changes in the Cell Organs of *Drosera rotundifolia* produced by feeding with Egg-albumen. *Quart. Journ. Micr. Sci.*, vol. 39, pp. 387–426, pls. 23–24.

'99. Further Studies in Drosera. *Quart. Journ. Micr. Sci.*, vol. 42, pp. 203–223, pl. 22.

KESSLER, L.

'71. Untersuchungen über die Entwicklung des Auges, angestellt am Hühnchen und Triton. Dorpat.

'77. Zur Entwicklung des Auges der Wirbelthiere. Leipzig, 112 pp., 6 pls.

KÖLLIKER, A.

'83. Zur Entwicklung der Auges und Geruchsorganes menschlicher Embryonen. *Verh. phys.-med. Ges. Würzburg*, n. s., vol. 17, pp. 229–257, pls. 10–13.

LIEBERKÜHN, N.

'79. Beiträge zur Anatomie des embryonalen Auges. *Arch. f. Anat. u. Phys.*, anat. abth., pp. 1–29, pls. 1–2.

LÖWE, L.

'78. Die Histogenese der Retina nebst vergleichenden Bemerkungen über die Histogenese des Centralnervensystems. *Arch. f. mikr. Anat.*, vol. 15, pp. 596–630, pl. 37.

MANZ.

'76. Entwicklungsgeschichte des menschlichen Auges. *Handb. ges. Augenheilk.*, Leipzig, vol. 2, pt. 2, pp. 1–57.

MARTIN, P.

'91. Zur Entwickelung der Netzhaut bei der Katze. *Zeitschr. f. vergleich. Augenheilk.*, vol. 7, pp. 25–41.

RAMON Y CAJAL, S.

'93. La rétine des vertébrés. *La Cellule*, pp. 121–260, 7 pls.

REAL Y BEYRO, M.

'85. Contribution à l'étude de l'embryologie de l'oeil. Paris, 143 pp., 4 pls. (Thèse.)

RETZIUS, G.

'81. Beiträge zur Kenntniss der inneren Schichten der Netzhaut des Auges. *Biol. Unters.*, vol. 1, pp. 89–104, pl. 1.

RIEKE, A.

'91. Ueber Formen und Entwickelung der Pigmentzellen der Chorioidea. *Arch. f. Ophthalm.*, vol. 37, pt. 1, pp. 62–96.

SCHENK, G. L.
'67. Zur Entwicklungsgeschichte des Auges der Fische. *Sitzb. Wien. Akad.*, vol. 55, pp. 480–492, pls. 1–2.
SCHULTZE M.
'67. Bemerkungen über Bau und Entwickelung der Retina. *Arch. f. mikr. Anat.*, vol. 3, pp. 371–382.
VASSAUX.
'88. Recherches sur les premières phases du développement de l'oeil chez le lapin. *Arch. d'Ophthalm.*, vol. 8, pp. 523–547.
WÜRZBURG, A.
'75. Beitrag zur Bildungsgeschichte der Iris und Retina beim Kaninchen. *Centralbl. f. med. Wiss.*, pp. 820–823.